IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Hammond et al.

Serial No: 09/866,557

Filed: May 24, 2001

For: Methods and Compositions for RNA Interference

Attorney Docket No. CSHL-P02-010

Art Unit: 1637

Examiner: Cynthia B. Wilder

Commissioner of Patents
Washington, D.C. 20231

Declaration Under 35 U.S.C. §1.132 of Frank McKeon

Sir:

I, Frank McKeon, of Boston, MA, hereby declare as follows:

1. I am a Professor of Cell Biology at Harvard Medical School. I arrived at Harvard Medical School in 1986 as an Assistant Professor, was appointed Associate Professor in 1991 and Professor in 1998. I received my undergraduate degree from Pomona College and my Ph.D. in 1984 from the University of California, San Francisco, where I also conducted postdoctoral research.

2. Through my research at Harvard Medical School, I have been involved in research directed to gene silencing using RNA Interference. In particular, my research has included the use of hairpin RNA to inhibit gene expression. I have submitted and published several scientific papers, most in peer-reviewed journals, pertaining to my research involving RNA interference.

3. I have reviewed the Hammond et al. U.S. Patent Publication No. 2002/0162126 (USSN 09/866,557, also herein the "Hammond Application"). I understand that the Hammond Application was filed May 24, 2001, and claims priority as a continuation-in-part to PCT Application No. PCT/US01/08435 (filed March 16, 2001) and U.S. Provisional Application Serial Numbers 60/189,739 filed March 16, 2000 and 60/243,097 filed October 24, 2000. Through my own research and review of scientific literature, I am aware of what the state of the art of relevant

RNA interference was at the time the Hammond Application and its priority applications were filed.

4. I have also reviewed the outstanding Office Action dated April 21, 2005, (herein the "Office Action") that was issued in connection with the Hammond Application, and the pending claims of the Hammond Application. I am aware that the pending claims cover methods for attenuating gene expression in mammalian cells suspended in culture using a transcribed hairpin RNA. I understand that the Examiner has rejected the pending claims on the grounds that the claimed subject matter was not described in the Hammond Application in such a way as to "reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention". I also understand that the Examiner has rejected the pending claims on the grounds that the Hammond Application does not provide sufficient guidance on how to make and/or use transcribed hairpin RNAs in mammalian cells without undue experimentation. I respectfully disagree with the Examiner's grounds for these rejections.

WRITTEN DESCRIPTION

5. I note that the application uses the terms "hairpin RNA" and "single self-complementary RNA", which a molecular biologist would understand to be interchangeable terms referring to hairpin RNAs. Moreover, it is apparent that the Hammond Application includes hairpin RNA species within the scope of the general term "dsRNA". For example:

- Paragraph No. 0037 states "*[t]he dsRNA construct may comprise one or more strands of polymerized ribonucleotide*". That paragraph goes on to state that "*[t]he double-stranded structure may be formed by a single self-complementary RNA strand or two complementary RNA strands*".
- Paragraph No. 0135 also states that the "*[t]he double-stranded structure may be formed by a single self-complementary RNA strand or two complementary RNA strands*".

6. As one means for introducing the hairpin RNA into a mammalian cell, the Hammond Application also clearly contemplates the use of expression systems that are intended to produce hairpin RNAs upon being transcribed in cells. To illustrate:

- Paragraph No. 0019 sates "*[y]et another aspect of the present invention provides a method for attenuating expression of a target gene in cultured cells, comprising introducing an expression vector having a "coding sequence" which, when transcribed, produces double stranded RNA (dsRNA) the cell in an amount sufficient to attenuate expression of the target gene, wherein the dsRNA comprises a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of the target gene*".
- Paragraph No. 0120 states "*[t]he dsRNA construct may be synthesized either in vivo or in vitro. Endogenous RNA polymerase of the cell may mediate transcription in vivo, or cloned RNA polymerase can be used for transcription in vivo or in vitro. For generating double stranded transcripts from a transgene in vivo, a regulatory region may be used to transcribe the RNA strand (or strands)*".
- Paragraph No. 0138 states "*[t]he dsRNA construct may be synthesized either in vivo or in vitro....For transcription from a transgene in vivo or an expression construct, a regulatory region (e.g., promoter, enhancer, silencer, splice donor and acceptor, polyadenylation) may be used to transcribe the dsRNA strand (or strands)*".
- Paragraph No. 0139 states "*[a] viral construct packaged into a viral particle would accomplish both efficient introduction of an expression construct into the cell and transcription of dsRNA construct encoded by the expression construct*".

In view of the definition of dsRNA, i.e., that it includes hairpin RNAs, a molecular biologist would conclude from these and other teachings of the Hammond Application that the inventors had intended to convey their invention included the use of expression constructs to produce, by transcription, hairpin RNA in cells. This is even more explicitly described in the Figures and Exemplification section which teaches a method for creating an expression construct for producing hairpin RNAs in cells. See e.g., Figure 27 and Example 3 titled "*A simplified Method for the Creation of Hairpin Constructs for RNA Interference*".

7. The Hammond application provides the necessary understanding of the mechanism by which RNA interference ("RNAi") works so that, in view of it's teachings and the state-of the-at in RNA Interference, one skilled in the art would appreciate that hairpin RNAs could be used to induce gene silencing in mammalian cells. The elucidation of the essential features of RNA Interference, which is explicitly provided by the teachings of the Hammond Application, was

the necessary step in the analysis of the RNA Interference phenomenon in order for one to realize that it was indeed a generalized phenomenon and that (i) it was conserved in mammalian and drosophila cells, (ii) it could be used in cells in culture; and (iii) that hairpin RNAs could be used in mammalian cells.

8. At the time of the earliest priority date of the Hammond Application, March 2000, procedures based on double stranded RNA-triggered silencing were fairly well-established tools for functional genomics of lower organisms (plants, invertebrates and fungi). See Jorgensen et al. (1998) Science 279: 1486; Waterhouse et al. (1998) Proc. Natl. Acad. Sci. 95:13959 and Wassenegger et al. (1998) Plant Mol. Biol. 37:349. Moreover, at that time there were indications in the literature that double-stranded RNA also had some ability to induce gene silencing in mammals. See, for example, Wianny et al. (2000) Nature Cell Biol 2:70 which showed that double-stranded RNA (in the form of two stranded duplex) could effect gene function in early mouse embryos.

9. As a result of biochemical and genetic approaches described in the Hammond Application, the mechanisms underlying RNA Interference in both invertebrates and vertebrates was elucidated to the point that I would have appreciated that hairpin RNAs could be used for inducing gene silencing in mammalian cells. In particular, the Hammond Application identified the existence of conserved machinery for double stranded RNA-induced gene silencing between drosophila to mammals. The Hammond Application also defined the RNA Interference pathway as proceeding via a two-step mechanism. In the first step, double stranded RNA is recognized by an RNase III family nuclease called Dicer, which cleaves the dsRNA into about 21-23-nt siRNAs (now called "small interfering RNA" or "siRNA" in the scientific literature) which the application refers to as "guide sequences". These guide sequences are incorporated into a multicomponent nuclease complex, termed "RISC" for RNA-induced silencing complex, which identifies substrates through their homology to the guide sequence and targets these cognate mRNAs for destruction. The key protein in the RISC complex is Argonaute, which, as described by the Hammond Application, is conserved between drosophila and mammals.

10. Example 1 of the Hammond Application shows the results of an experiment in which S2 cells were transfected with dsRNAs corresponding to either cyclin E or lacZ.

- These results indicate that RNA Interference can ablate target mRNAs in cultured cells through the generation of a sequence-specific nuclease activity. This enzyme activity was termed RISC.

- The Hammond Application also observed that the nuclease activity present in the extract could recognize the antisense strand of the target sequence, e.g. the target mRNA. Again, substrates that contained a substantial portion of the targeted region were degraded efficiently whereas those that contained a shorter stretch of homologous sequence were recognized. For both the sense and antisense strands, transcripts that had no homology with the transfected dsRNA were not degraded.

11. The Hammond Application showed that silencing provoked by dsRNA is sequence specific. For example, the application showed that pre-treatment of extracts with a Ca2+-dependent nuclease (micrococcal nuclease) abolished the ability of these extracts to degrade cognate mRNAs. Activity could not be rescued by addition of non-specific RNAs such as yeast transfer RNA. Although micrococcal nuclease can degrade both DNA and RNA, treatment of the extract with DNAse I had no effect. Sequence-specific nuclease activity, however, did require protein. I would hare reasonably interpreted these results to support a mechanism in which the RNAi nuclease is a ribonucleoprotein, requiring both RNA and protein components.

12. The biochemical fractionation described in Examples 1 and 2 of the Hammond Application is consistent with the RISC components being conserved between invertebrates and vertebrates, i.e., flies and humans.

- In plants, the phenomenon of co-suppression has been associated with the existence of small (25-nucleotide) RNAs that correspond to the gene that is being silenced. To address the possibility that a similar RNA might exist in Drosophila and guide the sequence-specific nuclease in the choice of substrate, the Hammond Application shows the partially purified activity through several fractionation steps. As described in Example 1, active fractions also contained an short RNA species that were homologous to the cyclin E target.

- Example 2 of the Hammond Application makes two important points, namely that the dicer enzyme was the nuclease responsible for the processing of double-stranded RNA to form guide sequences of about 22 nucleotides; and (ii) that the human dicer homolog was also capable of generating guide sequences of about 22 nucleotides. See also Figure 23.

The roles of a nuclease activity that cleaves regions of double-stranded RNA, the conservation of that nuclease activity and it's 22-mer products between drosophila and humans, and the ability of hairpin RNA to induce gene silencing, all taught by the Hammond Application, along with the state-of-the-art in using other double stranded RNA species to induce silencing, led me to conclude that the inventors had recognized and understood, and plainly articulated in their application, that part of their invention was the use of hairpin RNA to induce gene silencing in mammalian cells.

12. Indeed, the Hammond Application explicitly supports gene silencing in mammalian cells using the transcribed hairpin RNAs. For example, the Hammond Application provides that the disclosed methods for gene silencing are broadly applicable to a variety of cell types including mammalian cells such as primate and human cells. Additionally, the Hammond Application teaches various expression vectors, several of which are suitable for use in mammalian cells, for generating dsRNA species in cells.

13. For these reasons, I believe that a molecular biologist reading the Hammond Application would clearly conclude that the inventors had possession of, and had clearly described, the use of transcribed hairpin RNAs for gene silencing in mammalian cells. That is, the Hammond Application provides a clear written description of the use of hairpin RNAs for gene silencing in mammalian cells.

ENABLEMENT

14. The teachings of the Hammond Application, in light of the state of the art at the time the application was filed, clearly enable an ordinary molecular biologist to make and use transcribed hairpin RNAs for gene silencing in mammalian cells. In view of these teachings, no more than routine experimentation is required to select a target sequence in a mammalian gene to be silenced, design the hairpin motif that should include that sequence, generate an expression vector for transcribing the hairpin in a mammalian cell, and assessing whether or not the vector results in down-regulation of the level of expression of that gene, such as by reduced mRNA levels. Each step of this process involves routine molecular biology. In fact, hairpin RNA constructs are routinely designed and generated by graduate students in my laboratory at Harvard Medical School.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Frank McKeon, Ph.D.

Dated: July 29, 2005 Signature: